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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

In this research we extend and deepen our studies of the relation between dynamic structure and function in proteins. We study protein dynamics using flash photolysis together with near ultraviolet, visible, and near and mid-infrared spectroscopies over wide ranges in time (50ns-10ks), temperature (60-320K), and pressure (0.1-100MPa). Pressure is used both as a static thermodynamic variable and as a perturbation to study protein motions. Initially we study a simple biomolecular reaction - carbon monoxide (CO) binding to myoglobin (Mb). We have greatly expanded knowledge of pressure effects on proteins by using the infrared CO stretching frequencies in MbCO as a probe together with high pressure and low temperature. The combined pressure and temperature studies shed new light on various features of the hierarchical model of protein substates and motions. These studies also provide novel information on the glass-like behavior of proteins, including the slaved glass transition and glass-like relaxation processes near the transition temperature. We also extend our studies to more complicated proteins such as horseradish peroxidase and cytochrome P450.

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# PRESSURE STUDIES OF PROTEIN DYNAMICS

Hans Frauenfelder and Robert D. Young

Department of Physics
University of Illinois at Urbana-Champaign

Final Report

ONR Contract N00014-86-K-0270 R&T Code 4413015

## 1. SUMMARY OF RESEARCH GOALS AND METHODS

1.1 Overall Goals. We strive for an in-depth understanding of the relations between the dynamic structure and function of proteins. Our work over the last several years has shown conclusively that proteins are complex systems with a conformational energy landscape consisting of energy mountains separated by energy valleys (1,2,3,4). We refer to the energy valleys as conformational substates (CS). The CS of a protein have the same overall structure, but differ in detail (5,6); they perform the same function, but possibly at different rates (1,3). A protein can exist in many nearly isoenergetic substates so the ground state of a protein is quasidegenerate.



A small system such as a protein does not have sharp values of internal energy, entropy, and volume; these quentities fluctuate about their mean values. A resting protein does not remain in one CS, but fluctuates from one CS to another. A protein like myoglobin (Mb) has two different states – the liganded state such as carbonmonoxy-myoglobin (MbCO) and deoxy Mb. Equilibrium fluctuations (EF) are transitions among the various CS of the same protein state. The protein function – the transitions, MbCO ↔ Mb – is performed through functionally important motions (FIM). The FIM is a relaxation process (7,8,9). From the experimental point of view EF and FIM are related if the two types of motions explore the same, or at least similar, substates (2,3,4).

The conformational energy landscape of a protein may also be hierarchically arranged with valleys within valleys (2,3,7). Evidence is mounting that many, perhaps all, complex systems have a quasidegenerate and hierarchically arranged ground state. Examples of such complexity include glasses, spin glasses, evolution, neural networks, and the traveling salesman problem. Proteins may be models for other systems and a profound understanding of protein dynamics may impact on these other fields. Conversely deep theoretical knowledge of spin glasses, for example, may also deepen the understanding of proteins. Certainly understanding of the dynamic structure-function relationship in biomolecules will yield rich dividends in all the biological sciences, from marine biology to medicine and pharmacology. It is also possible that such an understanding will lead to advances in biotechnology including biomaterials, biosensors, and possibly bioelectronics.

1.2 Pressure Effects in Proteins. The effects of hydrostatic pressure on protein reactions and motions have been relatively unexplored. Most of the previous work on protein dynamics has been performed by varying temperature, but pressure is usually kept constant. Some work has been performed at high pressure, but this has usually been done at constant temperature or over a small temperature interval (10,11). However, pressure and temperature are on an equal footing as thermodynamic variables and, ideally, studies of protein reactions and motions should be performed by varying both temperature and pressure over wide ranges. While such studies are difficult experimentally, we had made some attempts prior to our ONR contract to do such experiments (12,13,14,15). The work during the last three years of our ONR contract has shown that novel and important data can result from combined pressure-temperature studies.

Pressure is used in two ways in our work (7,16): (i) In quasistatic experiments we determine the effects of hydrostatic pressure on various protein properties including protein spectra in the Soret, near infrared, and infrared wavelength regions. These measurements are performed on various proteins over a wide range of temperature and solvents. (ii) In

kinetic (relaxation) studies pressure is used as a perturbation to probe protein motions. In particular we observe the relaxation of the protein after a pressure release by monitoring protein spectra.

1.3 Functional Tool. The functional tool in our work is the binding of small ligands such as dioxygen ($O_2$) or carbon monoxide (CO) to heme proteins. We typically begin our studies using CO binding to Mb as a bench mark and to develop concepts and principles. We then move to other ligands and more complicated or mutant (both wild type and synthetic) heme proteins in order to probe the effects of protein structure. Eventually we study even more complicated membrane proteins such as bacteriorhodopsin.

1.4 Experimental Tools. We summarize our experimental and computational systems:
(i) Computational Tools. We have two DEC PDP-11 minicomputers for data acquisition and analysis. We also have a VAX station 2000 for more extensive "number crunching" and theoretical modelling. All of our computers are networked with the VAX computers at the University of Illinois and the CRAY XMP and CRAY 2 at the National Center for Supercomputing Applications.
(ii) Pressure Systems. Our basic pressurizing system uses nitrogen gas to reach pressures up to 270 MPa (30). Our current pressure cell uses single crystal sapphire windows and is rated for up to 200 MPa. We have designed and built a new pressure cell with considerably less thermal mass which will still allow pressures up to 200 MPa. The new pressure cell will allow for considerably smaller temperature equilibration times. The pressure system can be used with each of our experimental set-ups, including our OLIS Cary-14 spectrophotometer and our flash photolysis system for fast (10 ns to 300 ks) transient kinetic studies in the near ultraviolet to mid-infrared.
(iii) FTIR System. Measurements are taken on a state-of-the-art Mattson Sirius 100 FTIR spectrometer. Temperature is controlled with a closed-cycle helium refrigerator. Photolysis is obtained with a Phase-R D12100C dye laser using rhodamine 6G (pulse

width 500 ns, 0.3 J). Kinetic spectra are obtained from about 10 s to over 10 ks. This is the main experimental system for our quasistatic and kinetic pressure experiments.

(iv) Fast IR System. This system is a mid-infrared flash photolysis system with the monitoring light produced by a Spectra-Physics/Laser Analytics tunable-diode laser and detected by a liquid nitrogen-cooled HgCdTe detector. Photolysis is obtained with the same laser as the FTIR system. Temperature control is accomplished with a second refrigerator. Time range extends from about 100 ms to 100 s. The diode laser current is modulated at 10 kHz and the signal is amplified with a PAR 5101 lock-in amplifier.

(v) Fast (20 ns - 3 ks) flash photolysis system (380-800 nm). This system utilizes a frequency-doubled Nd:glass laser of 25 ns pulse width delivering 300 mJ of 530 nm light for photolysis. A 50 MHz transnsient digitizer (LeCroy TR8818) and home-built logarithmic time-base digitizer (1 μs to 3 ks in a single sweep) capture signals. Monitoring is done by a quartz-halogen light source or a CW dye laser (Spectra-Physics) from the Soret to near infrared. Temperature control is maintained with a Janis Varitemp dewar from 2K to 350K.

(vi) OLIS-Cary 14 Spectrophotometer. This instrument is interfaced to an IBM PC-AT for instrument control, data acquisition, and data analysis. Monitoring wavelength extends from the near ultraviolet to the near infrared. Kinetics data has a time range from 60 s to 50 ks. A continuous flow dewar permits temperature control from 5K to 350K.

1.5 Specific Goals. There are several goals which we have pursued during the ONR contract period including studies of the following phenomena: (i) The structure of the conformational energy landscape of proteins. (ii) The rules for relaxation and fluctuation of proteins in the conformational energy landscape. (iii) The dependence of the conformational energy landscape on protein structure.

## 2. MAIN RESULTS

The experiments conducted during our ONR contract have revealed a wealth of novel and stimulating results which are crucial for a deeper understanding of protein dynamics and function. In fact the results have exceeded our original expectations when applying for our ONR contract and have demonstrated the vital importance of pressure both as a thermodynamic variable in protein dynamics and as a tool for probing protein properties. Here we summarize the most significant results and the overall thrust of our work.

2.1 Concepts. The search for general concepts is a central goal of our work. We believe that these concepts are valid not only for the specific proteins which we study but for all biomolecules. Here we summarize several of the most significant concepts which we have developed during the period of the ONR contract: (i) Nonexponential time dependence of protein reactions and relaxations. Our previous work over more than a decade has established that the rebinding of small ligands to heme proteins after photodissociation at low temperature is nonexponential in time and can be approximated by a power law (1,3,4). During the last three years we have found that relaxation processes in myoglobin are also nonexponential in time (3,7,8,9,16). These observations show that proteins are complex systems and that this complexity is essential for protein function. (ii) Conformational substates. The nonexponential time behavior of rebinding at low temperature leads to the concept of conformational substates in proteins (1,3). The CS correspond to the local minima in the multivalley conformational landscape of the protein. (iii) Multivalley conformational energy landscape. A protein has a conformational energy landscape with many energy valleys separated by energy mountains and ridges. That is, the energy landscape of a protein is very rough. The energy valleys correspond to the conformational substates (CS). (iv) Hierarchy of conformational substates. The conformational substates are arranged in a hierarchy with the conformational energy landscape containing valleys within valleys. (v) Conformational labelling. MbCO exists in

at least three conformational substates which can be characterized by the properties of the CO infrared stretch bands, $A_0$, $A_1$, and $A_3$, such as peak frequency $\nu_i$, areas $A_i$, widths $\Gamma_i$, and tilt angles $\alpha_i$. (vi) Slaved glass transition in proteins. The quasistatic and kinetic experiments on Mb have shown that Mb experiences a glass transition which is slaved to motions of the solvent.

2.2 Conformational Substates of Tier 0. In myoglobin experiments suggest a hierarchy of CS in which the CS are arranged in at least four tiers (2,3,4,7,9). Studies under the ONR contract have clearly demonstrated the existence of two the four tiers of CS – tier 0 ($CS^0$) and tier 1 ($CS^1$). Tier 0 contains three or possibly four substates, denoted by $A_0$ to $A_3$, characterized by the infrared CO stretch bands. The $A_i$ satisfy the conditions for CS: They perform the same function, binding of CO, but with different rates (3). They correspond to a slightly different protein structure since the tilt angle $\alpha_i$ between the heme normal and the bound CO is different (17). They also have different energies, entropies, and volumes (3,16).

2.3 Conformational Substates of Tier 1. Rebinding of CO to each of the $A_i$ after photodissociation is nonexponential in time below about 180 K (3). The $A_0$ stretch band of the CO shifts to higher frequency with no change in intensity after pressure release at temperatures between about 165 K and 185 K (7). The infrared CO stretch bands also show kinetic (40 K) and spectral (10 K) hole burning (18). The simplest and essentially unique explanation is the existence of conformational substates of tier 1.

2.4 Slaved Glass Transition in Myoglobin. The multivalley conformational energy landscape is proteins implies analogies between proteins and amorphous solids like glasses and spin glasses. As the temperature of the protein ensemble is lowered or the viscosity of the solvent is increased, the proteins may not have sufficient thermal energy to cross the energy mountains in the energy landscape. The protein then experiences nonergodicity (19) and possibly a glass transition. Using FTIR spectroscopy to monitor the CO infrared

stretch bands in MbCO and differential scanning calorimetry, we have proven that Mb does indeed experience a glass transition which is slaved to motions of the solvent, in particular, the glass transition in tier 1 is slaved to the $\alpha$ relaxation of the solvent (3,7). We, therefore, refer to this transition as a slaved glass transition. Tiers 0 and 1 also appear to have slightly different slaved glass transition temperatures $T_{sg}$. For example, in 75% glycerol/water solvent $T_{sg}$ for tier 0 is about 195 K while $T_{sg}$ of tier 1 is about 178 K.

2.5 Protein Relaxations, FIM 0 and FIM 1. The existence of a hierarchy of CS in myoglobin implies a range of relaxations processes. We have probed relaxation processes in tiers 0 and 1 using time-resolved FTIR spectroscopy and pressure release for temperature near the slaved glass transition in MbCO in 75% glycerol/water (7,9,16). The relaxation process FIM 1 is characterized by the shift in the peak frequency $\nu_0$ of the $A_0$ infrared stretch band and is interpreted as a rearrangement of the substates of tier 1. The shift in peak frequency $\nu_0$ after a change in pressure from 100 MPa (1 kbar) to 7 MPa can be resolved into two processes, a rapid (elastic) change and a slower (plastic) relaxation. The elastic change is fast perhaps to as low as 10 K and may be identified with the elastic deformation found by Go (20). The plastic deformation (FIM 1) corresponds to relaxation within tier 1. FIM 1 has two characteristic features: (i) It has a nonexponential time dependence which is best described by a power law but which is also consistent with a stretched exponential (21). (ii) The temperature T dependence is described by a characteristic rate k(T) which can be parameterized by an Arrhenius law $k(T) = A\exp(-E/k_BT)$ where $E \simeq 1.5$ eV and $A \sim 10^{40}$ $s^{-1}$. Such an unphysically large preexponential A near the glass transition is well known from the $\alpha$ relaxation in glasses and implies that the relaxation is a cooperative phenomenon. The $\alpha$ relaxation has been parametrized by the three-parameter Vogel-Tamman-Fulcher relation (21). We use the two-parameter Bassler-Zwanzig relation $k(T) = k_0 \exp[-(T_0/T)^2]$ which also fits k(T) in glasses

over typically ten orders of magnitude. Similar results follow for FIM 0, characterized by the change in relative intensities of the CO infrared stretch bands $A_0$ and $A_1$.

The two relaxations observed at 200 K, FIM 0 and FIM 1, differ significantly: FIM 0 is slower than FIM 1, resulting in the higher $T_{sg}$ for FIM 0. FIM 0 and FIM 1 can be extrapolated to physiological temperatures with the Bassler-Zwanzig relation with the result that $k(300\ K) \approx 10^7\ s^{-1}$ for FIM 0, and $k(300\ K) \approx 10^{11}\ s^{-1}$ for FIM 1. The faster relaxation process FIM 1 is interpreted as a rearrangement of substates of tier 1 corresponding to a large-scale motion of the protein, but without an overall change in protein shape. The slower relaxation FIM 0 is interpreted as the transition from one substate of tier 0 to another. FIM 0 also involves a large-scale motion of the protein possibly with the entire protein slightly changing shape.

2.6 Extension to Other Heme Proteins. We believe that the general concepts discussed above for Mb also hold for all biomolecules. We have, therefore, begun to perform both quasistatic and kinetic experiments on more complicated heme proteins such as horseradish peroxidase type C (HRP-C). This protein also binds CO to the heme iron so that conformational labelling can be used to study any glass transition and protein relaxation process. Our previous work with HRP-C had shown evidence of interesting conformational changes not observed in Mb or monomeric heme proteins (22). The preliminary results from the current experiments are very exciting: The CO stretch bands of HRP-C-CO exhibit A bands which were not previously resolved. We also see evidence for a glass transition in the protein and a complex spectrum of relaxation processes. We will present a preliminary report of our results to the March (1989) Meeting of the American Physical Society (23).

We also have begun preliminary studies of the heme protein cytochrome P450 which also binds CO (24). In fact P450-CO has at least five CO stretch bands possibly

corresponding to five substates of tier 0. We expect to be able to study the slaved glass transition and FIM 0 and 1 in much more detail in P450 and HRP-C.

2.7 Biology and Physics. The techniques and the results of our ONR contract may be important both for physics and biology. The pressure jump approach together with site-specific spectroscopic observation permits site-specific studies of relaxation phenomena in amorophous systems. Genetic engineering yields controlled molecular modifications in proteins and hence permits relaxation studies on specifically designed systems (25). Proteins may well become paradigms of complex systems in physics. For biology, detailed knowledge of protein motions at different time and length scales is necessary for an understanding of protein and enzyme reactions at the molecular level. Slaving may be in fact an efficient control mechanism in cells and membranes.

## 3. New Equipment and Techniques

We continue to improve and develop our experimental set-ups: (i) We have pioneered the use of conformational labelling and time-resolved FTIR spectroscopy at low temperature and high pressure. We expect that these techniques together will yield a vast amount of crucial information regarding the dynamic structure and function of proteins. We also expect that this technique can be adapted for study of hydrogen-bonded glasses like glycerol and propanol near the glass transition and, therefore, could yield insight into the nature of the glass transition. (ii) We have developed a new high pressure-low temperature cell with significantly lower thermal mass, resulting in shorter temperature-equilibration times. This cell also allows us to extend our measurements to 10 K with our existing refrigeration system.

## 4. References and Publications

[Note: A single asterisk * denotes a publication based on work partially supported by this contract.]


1. Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H. & Gunsalus, I. C., Biochemistry **14**, 5355 (1975).
2. Ansari, A., Berendzen, J., Bowne, S. F., Frauenfelder, H., Iben, I. E. T., Sauke, T. B., Shyamsunder, E. & Young, R. D., Proc. Natl. Acad. Sci. USA **82**, 5000 (1985).
* 3. Ansari, A., Berendzen, J., Braunstein, D., Cowen, B. R., Frauenfelder, H., Hong, M. K., Iben, I. E. T., Johnson, J. B., Ormos, P., Sauke, T. B., Scholl, R., Schulte, A., Steinbach, P. J., Vittitow, J. & Young, R. D., Biophys. Chem. **26**, 337 (1987).
* 4. Frauenfelder, H., Parak, F. & Young, R. D., Ann. Rev. Biophys. Biophys. Chem. **17**, 451 (1988).
5. Frauenfelder, H., Petsko, G. A. & Tsernoglou, D. Nature **280**, 558 (1979).
* 6. Frauenfelder, H. *The Debye-Waller Factor: From Villain to Hero in Protein Crystallography*, in Int. J. Quant. Chem., in press.
* 7. Iben, I. E. T., Braunstein, D., Frauenfelder, H., Hong, M. K., Johnson, J. B., Luck, S., Ormos, P., Schulte, A., Steinbach, P. J. & Xie, A. H., Phys. Rev. Lett. submitted (1989).
* 8. Young, R. D., *Glassy Dynamics in Proteins*, in *Cooperative Dynamics* in *Complex Physical Systems*, Proceedings of the 2nd Yukawa Intl. Seminar, Kyoto, Springer-Verlag, in press.
* 9. Frauenfelder, H., Steinbach, P. J. & Young, R. D., Chemica Scripta, in press (1989).

10. Weber, G. & Drickamer, H. G., Q. Rev. Biophys. **6**, 89 (1983)

11. Jannasch, H. W., Marquis, R. E. & Zimmerman, A. M., Eds., *Current Perspectives in High Pressure Biology* (Academic Press, NY) (1987).

12. Eisenstein, L. and Frauenfelder, H., in *Frontiers of Biological Energetics*, Vol. 1, 680, Academic Press (1978).

13. Yue, K. T., Ph.D. dissertation, University of Illinois at Urbana-Champaign (1983).

14. Shyamsunder, E., Ph.D. dissertation, University of Illinois at Urbana-Champaign (1986).

15. Young, R. D., in *Structure, Dynamics, and Function of Biomolecules*, Eds., Ehrenberg, A., Rigler, R., Graslund, A., Nilsson, L., Eds. (Springer, NY) (1987).

* 16. Iben, I. E. T., Ph.D. dissertation, University of Illinois at Urbana-Champaign (1988).

17. Ormos, P., Braunstein, D., Frauenfelder, H., Hong, M. K., Lin, S.-L., Sauke, T. B. & Young, R. D., Proc. Natl. Acad. Sci. USA **85**, 8492 (1988).

18. Frauenfelder, H. & Ormos, P., in *Biological and Artificial Intelligence Systems*, Eds. E. Clementi (ESCOM Press) (1988).

19. Palmer, R. G., Adv. Phys. **31**, 669 (1982).

20. Go, N. Chemica Scripta, in press (1989).

21. Dorfmüller, Th. & Williams, G., Eds. *Molecular Dynamics and Relaxation Phenomena in Glasses*, Vol. 277, Lecture Notes in Physics, Springer-Verlag (1987).

22. Doster, W., Bowne, S. F., Frauenfelder, H., Reinisch, L. & Shyamsunder, E., J. Mol. Biol. **194**, 299 (1987).

* 23. Scholl, R., Braunstein, D., Frauenfelder, H., Hager, L. P., Hong, M. K., Johnson, J. B., Suh, Y. J., Xie, A. H. & Young, R. D., Bull. Am. Phys. Soc. **34**, 417 (abstract) (1989).

24. Berendzen, J. & Frauenfelder, H. (1988) in press.

25. Braunstein, D., Ansari, A., Berendzen, J., Cowen, B. R., Egeberg, K. D., Frauenfelder, H., Hong, M. K., Ormos, P., Sauke, T. B., Scholl, R., Schulte, A., Sligar, S. G., Springer, B. A., Steinbach, P. J. & Young, R. D., Proc. Natl. Acad. Sci. USA **85**, 8497 (1988).

* 26. Young, R. D., in *The Time Domain in Structural and Surface Dynamics*, Kluwer Acad. Publ., Boston, 107 (1988).

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INVESTIGATORS

Dr. Rodney L. Biltonen
Dept. of Pharmacology
University of Virginia
Charlottesville, VA 22908

Dr. P. A. George Fortes
Department of Biology, C-016
University of California
La Jolla, CA 92093

Dr. Hans Frauenfelder
Loomis Laboratory
University of Illinois
1110 West Green Street
Urbana, IL 61801

Dr. Perry M. Hogan
Department of Physiology
State University of New York
Buffalo, NY 14214

Dr. Thomas F. Murray
College of Pharmacy
Oregon State University
Corvallis, OR 97331-3507

Dr. Richard B. Philp
Dept. of Pharmacology & Toxicology
The Univ. of Western Ontario
Medical Sciences Building
London, Ontario, Canada N6A 5C1

Dr. Shalom R. Rackovsky
Department of Biophysics
University of Rochester
School of Medicine and Dentistry
602 Elmwood Avenue
Rochester, NY 14642

Dr. Lou Reinisch
Laser Biophysics Center
F. Edward Hebert School
of Medicine
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Joseph F. Siebenaller
Dept. of Zoology and
Physiology
Louisiana State University
Baton Rouge, LA 70803-1725

Dr. Anne Walter
Dept. of Physiology and
Biophysics
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